REMARKS

Claims 1, 12, 16 and 26 stand rejected under 35 USC §112, first paragraph, as not enabling for claims broadly drawn to any oilseed plant producing the claimed levels of EPA, DPA or DHA and comprises at least one desaturase gene and at least one elongase gene. It was further stated on page 3 of the Office Action that ". . . no evidence, in the form of published articles or other printed literature, has been presented to support this asserted characteristic of borage and other oilseed crops. Moreover, it is unclear whether all of the crops listed, particularly blackcurrant, are considered 'oilseed' crops. . . ."

Submitted herewith are printouts from Wikipedia, a free online encyclopedia. Attention is kindly invited to page 2 of the Borage printout under the heading, Characteristics and uses. It states that Borage (*Borago officinialis* L.) ". . .was cultivated for culinary and medicinal uses, although today commercial cultivation is mainly as an **oilseed**. . . ." (Emphasis added).

Submitted herewith is a discussion about borage oil does. This was printed out from the eVitamins web site. It also discusses black currant seed oil.

Attention is invited to the very top of page 3 of the Wikipedia blackcurrant printout. Blackcurrant (*Ribes nigrum*) is a species of Ribes berry native to central and northern Europe and northern Asia. It is also known as French "cassis." Blackcurrant seed oil is also rich in many nutrients, especially gamma-linolenic acid (GLA), an essential fatty acid.

Also included herewith is a copy of an excerpt, pages 1-24, from "Oil Crops of the World, Their Breeding and Utilization", edited by G. Robbelen, R. Keith Downy and A. Ashri, published by McGraw-Hill in 1989. It is stated on page 2 under the heading Oilseed Production and Distribution that there "are many species of plants in the world whose oil can be used for human consumption. Lennerts (1984) names forty different oilseeds, but only a small number of such crops are significant to the world's food supply and trade (Table 1.1). . . ."

Sayanova et al., Phytochemistry 65: 147-148 (2004) (copy previously submitted) mentions borage in Table 1 on page 152 and references another Sayanova article from 1997 which can be found in column 2 on page 157 in which borage oil was studied, specifically, "Expression of a borage cDNA containing an N-terminal cytochrome b5 domain results in the accumulation of high levels of delta-6-

desaturated fatty acids in transgenic tobacco. Proc.Natl. Acad.Sci. USA 94, 4211-4216.

It was stated at the bottom of page 3 of the Office Action that "the claims do not specify that the elongase correspond to at least one of the desaturases present, e.g., a delta-6 elongase and a delta-6 desaturase, wherein such correspondence appears to be required for proper substrate processing."

Attention is kindly invited to the previously submitted copy of Dr. Anthony Kinney's Declaration dated March 23, 2007, a previously submitted copy of a paper co-authored by Dr. Kinney and Dr. Howard Damude (both co-inventors of the claimed invention) and a previously submitted copy of a paper authored by Napier et al. All of these materials taken with the Roberts review demonstrate that a variety of approaches using different genes and combination thereof can be used to make transgenic plants producing long chain polyunsaturated fatty acids. Attention is kindly invited to Table 1 on page 105 of the Roberts review which summarizes a variety of genes, host plants, and reported LC-PUFA proportions found in seeds of these transgenic plants.

As was previously stated in the Response filed on November 19, 2007:

It should be noted at the outset that the pioneering work which constitutes the subject matter of this invention concerns placing a marine DHA and/or EPA biosynthetic pathway into oilseed plant and recovering significant amounts of DHA and/or EPA in the seed oil. **This was not known prior to Applicants' disclosure.**

Assuming *arguendo*, that it was possible to recreate these marine pathways in the seeds of land-based plants, it was not known what the endogenous "machinery" of a land-based plant would do with these pathways. It was not known if a resulting LCPUFA would be incorporated into the seed oil. Bear in mind that the endogenous machinery of land-based plants had **never** dealt with such fatty acids before, thus it was not clear to one of ordinary skill in the art as to what the outcome would be.

The outcome was unknown until Applicants demonstrated for the very first time in one oilseed plant. This is what is set forth in the above-identified application. Once this had been demonstrated, it can then be used in any oilseed plant that has comparable endogenous machinery. In other words, demonstration in one land-based oilseed plant that its endogenous "machinery" was capable of using the

LCPUFAs to make DHA and/or EPA is a demonstration with respect to any oilseed plant that has the same machinery, i.e., all oilseeds.

This is demonstrated quite clearly in the instant specification wherein an EPA pathway was added to another oilseed plant, Arabidopsis, and observing the resultant EPA appearing in the oil of the seed. Further support can be found in the research of Dr. Wu (discussed below) who added an EPA pathway to Brassica using the method disclosed in the specification. The fact that the level of expression in Brassica was not the same as in soybean or Arabidopisis is irrelevant since the level of expression achieved certainly fell within the parameters recited in Claim 1 :

> a transgenic oilseed plant that produces mature seeds in which the total seed fatty acid profile comprises **at least 1.0 %** of at least one polyunsaturated fatty acid having at least twenty carbon atoms and five or more carbon-carbon double bonds.

The Kinney Declaration discussed the Robert article which was discussed at the Feburary 13, 2007 interview. Attention was kindly invited to Table 1 on page 105 of the Robert article. This table summarizes genes, host plants and reported LC-PUFA proportions in seeds of transgenic plants:

Column 1 references the work of Abbadi et al. (2004) with respect to flax.

Column 2 references the work of Kinney et al. (2004) (which constitutes the subject matter of the instant application.) with respect to soy.

Column 3 references the author's work in 2005 with respect to *Arabidopsis*; and

Column 4 reference the work of Wu et al. (2004 with respect to *Brassica/*rapeseed).

The Robert article then goes on to discuss Dr. Kinney's work in column 1 on page 106 (and also mentions the instant patent application and publication). The work of Wu et al. is discussed in column 1, second paragraph. The Robert article states that **Dr. Wu used Dr. Kinney's method to produce high levels of AA and EPA in rapeseed.** Specifically, a delta-17 desaturase was used to shunt a large amount of AA into the n-3 pathway at EPA. **Thus, Dr. Wu's work demonstrates**

that Dr. Kinney's method worked with respect to *Brassica*. Irregardless of the LCPUFA oil level, Dr. Wu did exemplify the use of Dr. Kinney's method to achieve expression of LCPUFAs in *Brassica*, **a different oilseed plant than soy**. LCPUFAs were recovered. This further shows that the specification is indeed enabling.

Furthermore, it is noted that a number of different EPA/DHA genes from different sources are presented in the specification to produce EPA/DHA oils in transgenic oilseeds. It should be clear from the specification that as long as a pathway is capable of producing DHA and/or EPA, then that pathway can be used to produce such fatty acids in seed oils.

Attention is kindly invited to Figure 1 of Damude (2007) (previously submitted). This figure depicts aerobic LCPUFA biosynthetic pathways in marine microbes. In other words, there are a number of pathways available that could lead to the same product. Attention is kindly invited to Damude et al. (2007) second column on the third page through column 2 on the fifth page. This section discusses a variety of fatty acid biosynthetic pathways that can be considered in engineering omega-3 LCPUFAs in plants (also depicted in Figure 1).

Thus, Dr. Kinney's declaration also was intended to demonstrate that a variety of genes (from different sources) and combinations thereof can be used to engineer production of omega-3 fatty acids in oilseed crops. The art cited by Applicant demonstrates that the instant invention is not limited to any particular combination of enzymes (and genes encoding them). The choice of genes will vary depending on which pathway is chosen, for example, whether a delta-6 or delta- 8 pathway is chosen to engineer expression of an LCPUFA in a oilseed plant. The Napier, Wu and Damude articles all support this point.

Accordingly, in view of the foregoing discussion and submissions herewith, withdrawal of the rejection of Claims 1, 12, 16 and 26 under 35 USC §112, first paragraph, is respectfully requested.

Claims 1, 12, 16 and 26 stand rejected under 35 USC §103(a) as being unpatentable over Knutzon et al (US6,075,183) in view of Abbott Laboratories (WO 02/08042), further in view of each of Mukherji et al. (US 7,211,656) or Browse et al. (US6,884,921), as stated on pages 8-10 of the April 1, 2008 Office Action for claims 1, 11-12, 16-18, 26-28 and 141.

At the outset, it should be noted that the instant application was filed non-provisionally on February 11, 2004 and claims the benefit of priority of a provisional application that was filed on February 12, 2003.

Qi et al. was published in June, 2004. It was noted on page 5 of the Office Action that Qi was published prior to publication of Applicant's PCT Application. Applicants kindly note that the earliest priority date to which the instant application is entitled is **February 11, 2003.** Thus, priority date of the instant application predates Qi et al. by well over one year.

Abbadi et al. (October, 2004) was published well after the February 12, 2003 priority of the instant application. The same is true of Robert et al. (2006) and Wu (2005).

The reason that the foregoing articles were cited by Applicants was to demonstrate what has happened in the wake of the pioneering contribution made by Dr. Anthony Kinney and his group in engineering oilseed crops to produce omega-3 fatty acids. **The February 12, 2003 priority date of the instant application demonstrates that Dr. Kinney and his team were at the forefront of engineering oilseed crops to produce omega-3 fatty acids.** (Emphasis added).

The basis of rejection of the claims under 35 USC §103(a) rejection as set forth in the Office Action dated April 1, 2008 was that it "would have been obvious to one of ordinary skill in the art to utilize the method of Brassica transformation with the delta-5 an delta-6 desaturase genes under the control of the seed-specific napin promoter for the production of novel PUFAs in the seed oil of transgenic plants as taught by Knutzon et al, and to modify that method by incorporating the elongase genes taught by ABBOTT LABORATORIES under the control of a seed-specific promoter,and to further modify that method by incorporating the omega-3/delta 17 desaturase genes taught by Mukherji et al or Browse et al under the control of a seed-specific promoter such as the napin promoter for the production of EPA in the oil of the transgenic Brassica seeds, as suggested by each reference."

Knutson describes the production of GLA and STA. The Abbott, Bioriginal and Browse patents disclose LCPUFA elongases and desaturases.
GLA and STA are found in the oils of some plants.

It is respectfully submitted that it would not have been obvious to combine the aforemeintioned references for the reasons set forth hereinbelow.

Synthesis of of GLA and STA is a single linear conversion from LA or ALA.

In contrast, EPA and DHA are membrane lipids not normally found in any plant oil. Synthesis of EPA from endogenous plant lipid is not linear. Prior to the instant invention, it was not known if a plant could accumulate these fatty acids in their oil.

Fatty acids are desaturated while part of membrane lipids, they are elongated while attached to acyl-CoA. Thus, fatty acids need to pass in and out of the phospholipids in the plant cell membrane as part of the pathway to synthesizing EPA. This step was thought to be a major block in converting ALA to EPA in plants (as described by Robert, copy previously submitted). Consequently, this led researchers to look for acyltransferases involved in the exchange of acyl groups between membrane lipids.

In Wu et al, cited by Robert , the investigators use the same method disclosed in the instant application to make 15% EPA in *Brassica* seeds. When an additional acyltransferase is added to the constructs, there appeared to be no effect on the total EPA content. Robert also describes an attempt by his own group to look for acyl-CoA desaturases from fish and express them in plants in an effort to avoid having fatty acids pass in and out of membrane phospholipids. It was concluded, however, that this is not necessary and, in the light the information disclosed in the instant application, should essentially use the methods described in the instant application to design LCPUFA pathways.

Likewise, the synthesis of DHA from EPA in higher organisms is a complex series of events involving two acyl-CoA elongation reactions, movement to membrane phospholipids, a membrane desaturation., movement of the resultant fatty acid into the peroxisome and then a beta-oxidation reaction to produce DHA.

Accordingly, it would not have been obvious to one of ordinary skill in the art that adding a delta-4 desaturase to transgenic seeds designed to produce EPA using the methods disclosed in the above-identified application, would lead to the formation of DHA given the complexity of the pathway.

Applicants respectfully request consideration of the possible rejoinder of claims 21 and 140 in the event that claim 1 is found to be allowable. Claims 21 and 140 depend from claims 1 or 12 and incorporates all the limitations thereof.

A notice of appeal accompanies the filing of this Response After Final.

Please charge any fees or credit any overpayment of fees which are required in connection with the filing of this Response to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company).

It is respectfully submitted that the claims are now in form for allowance which allowance is respectfully requested.

In view of the foregoing, allowance of the above-referenced application is respectfully requested.

Respectfully submitted,

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